

Characterization of Small Molecule Inhibitors of the Fanconi Anemia Pathway

C. Jacquemont¹, A.D. D'Andrea², T. Taniguchi¹

¹Fred Hutchinson Cancer Research Center, Seattle, WA; ²Dana-Farber Cancer Institute, Boston, MA

Objective: DNA-crosslinking agents including cisplatin are widely used for the treatment of tumors, but resistance to these drugs is a major limitation of the treatment. The integrity of the Fanconi anemia (FA) pathway is required for cellular resistance to DNA-crosslinking agents. Therefore, small molecules which inhibit this pathway are expected to sensitize cancer cells to DNA crosslinkers, and may be useful as chemosensitizers for the treatment of patients with DNA crosslinker-resistant cancer. We previously reported that curcumin, identified through a screen of approximately 5,500 chemicals for FA pathway inhibitors, sensitizes tumor cells to cisplatin (Chirnomas *et al*, Molecular Cancer Therapeutics, 2006). However, the size of the screened chemical library was rather small and characterization of other identified candidate inhibitors was incomplete. The objective of this study is to identify small molecule inhibitors of the FA pathway from larger chemical libraries, and to characterize their mechanism of action.

Methods: We screened more than 16,000 chemicals (including the 5,500 chemicals screened previously) using a high-throughput screening assay utilizing the inhibition of DNA damage-induced FANCD2 foci formation as a readout. We analyzed the effect of the identified FA-pathway inhibitors on the recruitment at sites of DNA damage of key DNA damage response proteins (FANCD2, BRCA1, RAD51, etc.) and on the efficiency of homologous recombination. The ability of these compounds to inhibit proteasome function was also analyzed, since we had found that the proteasome function is required for the activation of the FA pathway.

Results: Twenty-three chemicals inhibited IR-induced FANCD2 foci formation in multiple cell lines. These chemicals include 17 compounds with known bioactivities (4 proteasome inhibitors, 2 CDK inhibitors, 1 HSP90 inhibitor, curcumin, etc.), and 6 compounds without any known bioactivity. Most of the compounds also inhibited IR-induced BRCA1 and/or RAD51 foci formation, and decreased the efficiency of homologous recombination. In addition to 4 known proteasome inhibitors, curcumin and one of the compounds without known bioactivity exhibited proteasome inhibiting activity.

Conclusion: We identified 23 chemicals which inhibit the foci formation of FANCD2, including a novel proteasome inhibitor. Most of them inhibited homologous recombination, and therefore may sensitize tumor cells to DNA crosslinking agents.

Translational Applicability: This study may lead to the discovery of new drugs useful as chemo-sensitizers in cancer treatment. Elucidating the mechanisms of action of these drugs will further clarify the pathogenesis of FA and may eventually lead to new approaches of the treatment of patients with FA and/or cancer.